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# A reliable method for ageing of whiting (*Merlangius merlangus*) for use in stock assessment and management

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## ABSTRACT

Accurate age estimation is important for stock assessment and management. The importance of reliable ageing is emphasized by the impending analytical assessment of whiting (*Merlangius merlangus*) in the Baltic Sea. Whiting is a top predator in the Western Baltic Sea, and is fished commercially although less extensively compared to the North Sea. Even though the species is considered one of the most difficult gadoids to age, few efforts have been made to shed light on the ageing problems. The aim of the present study was to identify and validate the 1st winter ring and to examine the visibility of the subsequent winter rings. Microstructure analysis was used to confirm the 1st winter ring. Additionally, otolith growth trajectories were obtained, confirming the allometric growth seen in many fish species. The method for ageing of whole otoliths presented in this study can be directly implemented in future ageing of whiting otoliths from the Baltic Sea – and potentially also adjacent areas where the conspecifics have similar growth rates.

*Keywords:* age estimation, otolith growth, validation, microstructure analysis, assessment

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# Introduction

Whiting (*Merlangius merlangus*) is a commercially fished species throughout most of its distribution range. The increasing importance of the species in the North Sea and the Skagerrak is seen in the catches which have increased concomitantly with a decrease in catches of other gadoids such as cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) (Anon. 2012*a*/*b*). In the current EU directive regarding sampling of commercially important species, it is only obligatory to collect whiting in the North Sea, Kattegat and Skagerrak (EU 2010). Analytical assessment is, currently, only conducted for whiting in the North Sea.

Acknowledging that whiting is a top predator in the western Baltic Sea, it is important to investigate its role, i.e. life-history traits, ecology and population dynamics. Such information can be used in multispecies modeling and for potential future analytical assessment. This emphasizes the importance of correct age estimation since under- or overestimation will influence ecological studies and bias the assessment (Beamish & McFarlane 1983; Campana 2001; Reeves 2003; de Pontual *et al.* 2006). Whiting is considered to be a difficult gadoid to age (CEFAS 2005), and it is therefore essential to develop a reliable ageing method, which has potential application for other whiting stocks as well.

Vertebrae, scales, fin rays and otoliths are all used in ageing of fish, the latter being the primary method (Campana 2001; Campana & Thorrold, 2001). Routine ageing of otoliths is based on visual identification of growth zones (Campana & Thorrold 2001); an opaque zone is formed during the growth period (summer) and a translucent zone during periods of slow growth (winter). An annulus comprises both zones, but as 1st of January is set to be the birth date of all fish, only the translucent zones are counted when ageing (Pannella 1974; Smedstad & Holm, 1996).

Though commonly used, the traditional age estimation method has proven to be quite challenging in many gadoids such as Baltic cod (Hüssy 2010; Rehberg-Haas *et al.* 2012), European hake (Morales-Nin *et al.* 1998; de Pontual *et al.* 2006) and whiting (Polat & Gümüs 1996; CEFAS 2005). Validation is required to ensure correct and reliable ageing (Beamish & McFarlane 1983). The most appropriate method to validate the age of a fish species is by mark/recapture studies marking both fish and otolith. This technique, however, is very time-consuming (Beamish & McFarlane 1983; Polat & Gümüs 1996; Campana 2001). Methods for identifying the 1st winter ring and investigating the seasonality in ring pattern have been applied such as breaking or grinding of otoliths (Polat & Gümüs 1996), microstructure analysis (Hüssy 2010; Hüssy *et al.* 2010) or other methods (see review by Campana 2001).

The otoliths of whiting exhibit a similar annulus pattern as seen in many other fish species with a broad opaque zone forming during the growth season (spring-summer) and a narrow translucent zone during the period of reduced growth (winter) (Bowers 1954). As whiting grow larger, calcium carbonate is accumulated in the area around the nucleus, inhibiting the visibility of the 1st and possibly also 2nd winter ring. This has been observed in whiting in the North Sea (Gambell & Messtorff 1964), the Irish Sea (Bowers 1954) and the Black Sea (Polat & Gümüs 1996). In the latter study it was concluded that due to the thickness of the central area of the otolith, the risk of missing the 1st and 2nd annuli is high, hampering ageing based on whole otoliths. Problems relating to false winter rings, i.e. translucent zones formed during the year in response to changes in the environment, have additionally been reported for North Sea whiting (CEFAS 2005).

Different ageing methods such as grinding and breaking of the otoliths have been tested in whiting from other areas. Grinding of otoliths is a reliable but time-consuming method (Bowers 1954; Gambell & Messtorff 1964; Polat & Gümüs 1996). Breaking of otoliths is a useful method for ageing of younger whiting (Polat & Gümüs 1996; CEFAS 2005), but as the fish grow older, the ring pattern becomes increasingly difficult to distinguish due to decreasing distances between the annuli (Gambell & Messtorff 1964). These studies have primarily focused on finding the best age estimation method, although investigating the seasonality in the edge formation is part of the age validation process. The first step in the validation process is to identify and validate the 1st winter ring (Campana 2001). The next step is to investigate the seasonality in the edge zone formation and to explore the consistency of the annulus pattern (Campana 2001). Both steps should theoretically be carried out for all age classes and for different years (Beamish & McFarlane 1983), though this is often difficult to achieve (Campana 2001).

Using whiting from the Western Baltic Sea, the objectives of this study are (1) to confirm the previous findings on whiting otoliths, i.e. the increasing difficulties in distinguishing the first annuli with increasing fish size and the seasonality in zone formation, (2) to identify and validate the first annulus and (3) to show individual otolith growth profiles, which will shed light on the changes in otolith growth rate from juvenile to adult. Additionally, a smaller sample of otoliths from the North Sea was examined to investigate whether similar problems regarding the decreasing visibility of the 1st winter ring exist.

## Materials and Methods

### Sample selection

Whiting were caught randomly during the extended BITS surveys in November 2011, January and May 2012. Stratified random sampling according to ICES square and depth stratification was conducted in the Fehmarn Belt (with a standard TV3-520 bottom otter trawl, OTB) in the southern part of the ICES subdivision 22 (fig. 1). The whiting were measured to the nearest cm below, weighed and the sagittal otoliths removed.

Fish used for identifying the 1st annulus by microstructure analysis were selected randomly from the peaks in the 2009, 2010 and 2011 cohort length distributions (i.e. 0-3 group), respectively (fig. 2). A total of 60 fish were selected (20 fish per survey), covering a length range of 8-30cm. Fish belonging to the 2009-2010 year-classes were subsequently used to test whether the increment pattern in older fish was consistent with the patterns observed in the first year of the same cohort. However, as year classes of whiting from other areas have been shown to overlap in length ranges (Gambell & Messtorff 1964; Flintegaard 1980; Armstrong *et al.* 2004), the tails of each cohort's length distribution were also sampled. These fish were also used in the edge formation analysis and for examining the visibility of the 1st annulus. Additional 11 fish in the size range 30-36cm were included in the latter analysis to confirm that only the 1st winter ring "disappeared" in the larger fish.

No samples were available for the 3rd quarter in the Fehmarn Belt surveys. To investigate the seasonality in the otolith edge formation, additional samples were taken with midwater otter trawl (OTM) in the acoustic survey performed by the German vessel, R/V Solea in September 2011 in ICES subdivision 24 (fig. 1). 20 fish were randomly selected and otoliths from them only used for the edge formation analysis. Together with otoliths used for the identification of the 1st annulus, otoliths from a total of 80 fish were used in the edge formation analysis.

To test the applicability of this approach to other stocks, 15 otoliths from whiting in the North Sea were used in a separate analysis. Fish were randomly selected from a discard survey conducted with a multi-rig otter trawl (OTT, 90 mm mesh size) in June 2011 in the northeastern part of the North Sea (close to the Skagerrak). These fish covered a length range of 17-28 cm.

## Analyses

Otoliths were investigated using three different methods: (1) ageing of whole otoliths, (2) ageing of ground otoliths and (3) examination of daily increment patterns, i.e. detection of zones with relatively smaller increments (low growth) assumed to correspond to the formation of a winter ring. The analyses were based on the following assumptions: (1) one year's growth corresponds to an opaque and a translucent zone; (2) this pattern is consistent throughout the life of the fish; and (3) periods of slow and fast growth (i.e. during winter and summer) can be observed as zones of decreasing and increasing daily increment widths. All image analyses were carried out in IMAGE PRO (vs. 5.0) and for the statistical analyses the Statistical Software *R* (R Development Core Team, 2009) was used.

## Ageing of whole otoliths

The otoliths were placed in propylene glycol, sulcus facing upwards, and viewed under a stereomicroscope (Leica MZ12) at a 1.25x magnification corresponding to 2.56 µm pixel$^{-1}$ using reflected light in a standardized set-up. Images were digitized (Leica camera DFL290) using a standard set-up. The distance from the nucleus to the 1st annulus ($D_{Traditional}$) was measured on the anterior axis from the nucleus towards the tip of the rostrum (fig. 3).

## Ageing of ground otoliths

The otoliths were glued to a glass slide using thermoplastic resin (Buehler) and ground on both sides on a rotating disc with two different abrasive papers (grit 3 µm and grit 1200 µm) to a thickness of approximately 500 µm. The ground otoliths were viewed and treated according to the procedure above. The distance from the nucleus to the 1st annulus ($D_{Ground}$) was measured (fig. 3).

## Annulus pattern and individual growth trajectories

The consistency of the sequential annuli was investigated and it was further tested whether there was a correlation between the 1st visible annulus in the whole otoliths and the 2nd annulus in the ground otoliths. This was done by comparing the distance from the nucleus to the 1st winter ring ($D_{Traditional}$) in the whole otoliths with the distance from the nucleus to the 2nd winter ring ($D_{2Ground}$) in the ground otoliths. Similarly, the 2nd visible annulus in the whole otoliths was compared with the 3rd annulus in the ground otoliths

## Daily increment pattern and identification of the 1st annulus

Microstructure analysis of the daily increments generates a similar pattern as the yearly banding with translucent zones corresponding to the period of slow growth (usually during the night) and opaque zones corresponding to the period of fast growth (day). One increment is comprised of a translucent and an opaque zone. Microstructure analysis or marginal increment analysis (MIA) is a good method to identify and validate the 1st winter ring (Campana 2001). Increment widths should display a sinusoidal cycle when plotted against time, i.e. during winter the widths decrease and during summer they increase (Campana 2001).

The ground otolith sections were viewed under a microscope (Leica DMLB) at a 10x magnification corresponding to 0.46 µm pixel$^{-1}$ using reflected light in a standardized set-up. Images were digitized (QImaging QIcam Fast 1394) using a standard set-up. The daily growth increments were investigated using the "caliper tool" in IMAGE PRO (vs. 5.0) which generates a profile of grey values ranging between 0, black, and 255, pure white. The beginning of an increment was defined as the rising point of inflection between the previous opaque zone and the subsequent translucent zone and was calculated from the divergence of individual pixel grey values from the running average. The distance from the nucleus on progressing days $i$ was calculated as $\text{Distance}_i = \text{Distance}_{i-1} + \text{Increment}_i$. The $\text{Distance}_{i-1}$ was standardized to the anterior axis by multiplying with the ratio between the length of the anterior axis and the length of the axis used for increment measurements. The $\text{Increment}_i$ was standardized in a similar way, i.e. by multiplying with the ratio between the increment widths on the anterior axis and the increment widths on the axis used for increment measurements. Zones in which increments were difficult to distinguish were measured and added to the total distance, but leaving out the individual increments from the analysis. To reduce the inter-individual variation, the increment widths for each individual were standardized to the widest increment ($\text{increment}_i / \text{increment}_{\max}$).

This resulted in growth profiles (nucleus to edge) showing the increment widths in relation to the distance from the nucleus. The distance from the nucleus to the midpoint of the 1st zone with decreasing increment widths, $D_{Increment}$, was measured.

### Seasonal otolith edge formation

The otolith edge was investigated to determine when the formation of the winter ring is initiated and ended. Four months were chosen (January, May, September and November) and 20-30 otoliths were analyzed per month. The otoliths were ground in accordance with the procedure mentioned above and the edge of each otolith was inspected visually. Otoliths were categorized as having an opaque or translucent edge, respectively.

### North Sea otoliths

Ageing of whole and ground otoliths from the North Sea was conducted in a similar way as with the Baltic Sea otoliths. Only otoliths of fish above 16 cm were included in this analysis as problems regarding the visibility of the 1st winter ring do not arise until the fish reach a certain size (Bowers 1954; Gambell & Messtorff 1964; Polat & Gümüs 1996).

## Results

### Comparison of $D_{Traditional}$ and $D_{Ground}$

The central area of the whiting otolith is thick and the zones less distinctive (fig. 4a). Comparison of whole and ground otoliths showed that the first annulus becomes increasingly difficult to detect by traditional ageing of whole otoliths as the fish grow larger (fig. 4a). There was a significant difference between the values of the ageing of the whole otoliths and the ground otoliths (paired t-test, df = 70, $p < 0.001$). This is also seen when comparing the actual distances from nucleus to 1st annulus, $D_{Traditional}$ and $D_{Ground}$ (paired t-test, df = 68, $p < 0.05$).

The distance from nucleus to 1st annulus increases linearly with fish size up until a size of 16 cm in both whole and ground otoliths (fig. 5a). Thus, $D_{Traditional}$ and $D_{Ground}$ are not significantly different in fish < 17 cm (ANOVA, df = 65, p = 0.523). At fish lengths ≥17 cm, $D_{Traditional}$ continues to increase linearly with fish length, whereas $D_{Ground}$ stops at a threshold value of approximately 3600 µm. This gives an interval of 1800µm (~1800-3600µm) from the nucleus in which the 1st winter ring can be assumed to lie within.

## Comparison of $D_{Traditional}$ and $D_{2Ground}$

The 2nd annulus was not difficult to distinguish in the whole otoliths investigated in this study, i.e. the 1st visible translucent zone observed in the whole otoliths in the 2+ groups corresponded well with the 2nd translucent zone seen in the otoliths after grinding (ANOVA, df = 41, p = 0.579) (fig. 5b). Similarly when comparing the distance from the nucleus to the 3rd annulus in the ground otoliths with the distance from the nucleus to the 2nd annulus in the whole otoliths (ANOVA, df = 41, p = 0.860) and for the 4th annuli (ANOVA, df = 17, p = 0.823).

## Comparison of $D_{Ground}$ and $D_{Increment}$

The daily increment widths generated a dome-shaped pattern with increasing widths during summer, where the fish grows, and decreasing widths during winter, when growth is stalled (fig. 6a). The increment widths become very narrow, but they never disappear completely (fig. 6b).

By applying a fifth degree polynomial trend line to each growth profile, the midpoint of the area of consistently decreasing increment widths was identified visually and the distance from nucleus to the midpoint ($D_{Increment}$) was recorded. This area corresponded well with the formation of a translucent zone (fig. 6c). Additionally, there appears to be a juvenile/settling zone approximately 1500 µm from the nucleus, which should not be misinterpreted as the first winter ring (fig. 4b). This was confirmed by the microstructure analysis which showed continuously broad increment widths throughout the zone. A winter ring would have corresponded with a decrease in increment width. Based on the microstructure analysis, an interval for each winter ring could be obtained (table 1). The juvenile zone was only visible in the ground otoliths and it was narrower than the winter rings.

There was a high degree of consistency between $D_{Ground}$ and $D_{Increment}$ (ANOVA, df = 67, p = 0.905) (fig. 6a/c). Combining the two methods provides an estimate of the maximum distance from the nucleus to the 1st annulus, which was found to be 3600 µm.

## Growth trajectories

The otoliths from the larger fish all showed a consistent winter ring pattern with decreasing distances between the annuli (fig. 7, table 1). The range of each winter ring was large, indicating that significant variation in otolith growth exists (table 1). Additionally, the ranges overlapped, i.e. the upper limit for the 1st annulus was ~3600µm and the lower limit for the 2nd annulus was ~2900µm (table 1, fig. 5).

## Seasonal otolith edge formation

The development in the opacity of the edge zone followed a seasonal pattern, although differences between otoliths existed. It was difficult to determine the degree of opacity of otoliths sampled in May and November as these months are part of a transition period in which the growth is either increased or reduced. Optical effects as well as the thermoplastic resin, in which the otoliths lay in, further complicated the interpretation. Most of the otoliths analyzed in January were observed to have a translucent edge, whereas in May approximately 30% had a translucent zone (fig. 8). In September, only a very small fraction had a translucent zone and this fraction increased in November to approximately 70% (fig. 8).

## North Sea otoliths

Comparison of whole and ground otoliths did not show a similar consistent problem in relation to distinguishing the 1st winter ring (paired t-test, df = 14, p = 0.334). The distance between the nucleus and the 1st annulus was not significantly different (paired t-test, df = 14, p = 0.216). Nevertheless, the opaque zones were difficult to distinguish in two out of the fifteen otoliths, and grinding was necessary to ensure that the ageing was correct.

# Discussion

This study confirmed earlier studies which have shown that traditional ageing of whiting otoliths is challenging and may result in underestimation of age (Gambell & Messtorff 1964; Polat & Gümüs 1996). In order to validate the winter ring formation, three issues were addressed: (1) seasonality of the otolith edge formation, (2) identification of the 1st winter ring and examination of the visibility of the 2nd and succeeding winter rings, and (3) individual otolith growth trajectories. Additionally, otoliths from North Sea whiting were examined to test the applicability of the present approach to other stocks.

Beamish & McFarlane (1983) were very strict about the validation of all ages and stated that extrapolation beyond the maximum validated age between populations can result in serious errors. They also pointed out that the only correct validation method is by mark/recapture. Considering the temporal scale of such a validation study as well as the fact that a large amount of the tagged fish would end up in the fishing nets during the first few years, the approach presented in this study is a valid substitute. The difficulties of obtaining whiting with known ages were also stressed by CEFAS (2005). With regard to validation of all ages, it was considered reliable to focus on identifying the 1st winter ring as the main issue in whiting appears to be the increasing thickness of the core area of the otolith with age, inhibiting the visibility of the 1st winter ring in whole otoliths (Bowers 1954; Gambell & Messtorff 1964; Polat & Gümüs 1996). This conclusion was supported in the present study which showed that from the 2nd winter ring and onwards, the annuli were always visible.

In accordance with earlier studies (Bowers 1954; Gambell & Messtorff 1964), the edge formation was found to vary over the seasons with most otoliths having a translucent edge in the winter and early spring. Thus, one of the main requirements put forth in the beginning of the study is fulfilled, i.e. the synchronous appearance across all individuals of an opaque and a translucent zone corresponding to fast and slow growth, respectively.

The microstructure analysis showed a consistent pattern with increasing increment widths in the period corresponding to summer, where the temperatures are high, and decreasing increment widths during winter (fig. 6a). This pattern persisted in the 2nd and 3rd year of life in the otoliths studied, and is thus considered to be representative for all year-classes. The observed increment pattern is seen in other gadoids like Baltic cod (Hüssy 2010; Hüssy *et al*. 2010), haddock and saithe (Quiñonez-Velázquez 1998), but also in other fish species, e.g. Atlantic herring (Clausen 2006; Oeberst *et al.* 2006), boarfish (Hüssy *et al.* 2012), and sprat (Baumann *et al.* 2006).

The increment widths became successively narrower during winter, but never ceased completely as in eastern Baltic cod (Hüssy 2010; Hüssy *et al*. 2010), North Sea herring (Clausen 2006) and boarfish (Hüssy *et al.* 2012), where the increments disappear during winter concurrently with the formation of the translucent zone. The reason for the continuous increment formation in Baltic Sea whiting is not known and analyses of the increment pattern in older fish as well as in whiting from adjacent areas should be conducted to investigate this further.

The 1st annulus was identified in 0 to 3-group fish by applying microstructure analysis which confirmed the first translucent zone to be a winter ring associated with low water temperatures.

In some of the otoliths a translucent zone approximately 1500 µm from the nucleus was observed. Though the zone appeared translucent, no concurrent decrease in increment widths was observed, and the zone was thus not considered to be an annulus. This juvenile/settling zone may be similar to the one found in whiting from the Irish Sea and the North Sea, referred to as the Bowers' zone, which is formed during late summer and likely relates to the change from pelagic to demersal habitat (Bowers 1954; Gambell & Messtorff 1964). It was generally easy to distinguish from the translucent zone formed during the first winter as the juvenile zone appeared close to the nucleus (~1500 µm). Bowers (1954) also noted that the translucent zone is much narrower than the actual annulus and this was also confirmed in the present study. More importantly, the microstructure analysis confirmed that this zone was not a winter ring since no decrease in increment widths was observed. The juvenile zone is only visible in ground otoliths, where microstructure analysis may reveal its nature.

The distance from the nucleus to the 1st winter ring showed large variation, the same applied to the succeeding winter rings (table 1). Whiting is a batch spawner, and in the North Sea and the Irish Sea, the species have been reported to spawn over an extended period (February to September) (Bowers 1954; Gambell & Messtorff 1964; Hislop 1975; Cohen *et al.* 1991), hence it does not seem unreasonable to have a large variation in otolith growth, i.e. larvae hatched late in the season will have a significantly reduced growth season. The 1-group fish (2011 cohort) used in this study ranged in size from 8-20 cm with the smallest fish being caught in May.

Whiting from the present study were capable of growing up to 20 cm within the first year of life. The rapid growth was also confirmed by the otolith growth trajectories which showed large otolith growth during the first year and then decreasing growth in the succeeding years (fig. 7). Bowers (1954) noted that from the second year and onwards, the growth is more moderate, i.e. 5-6 cm per year in Irish Sea whiting.

In most marine fish species, the initial growth is determining for growth later in life, hence a fish with a low growth rate in the first year will usually have slow growth throughout its life span

(Krohn & Kerr 1997; Armstrong *et al*. 2004; Rindorf 2008). This was also seen in the present study, where fish with the largest initial otolith growth generally achieved the overall largest otolith growth (fig. 7), corresponding to the highest length-at-age. The decrease in otolith growth with age is in agreement with the allometric growth seen in most species, especially after maturation where a proportion of the energy is allocated towards reproduction (Björnsson & Steinarsson 2002).

The fact that the ranges of the winter rings overlapped (table 1) was not surprising considering the large variation in hatching time and the resulting overlap in length distributions for the different year-classes. The large variation in length-at-age is also seen in whiting from other areas (Bowers 1954; Gambell & Messtorff 1964; Flintegaard 1980; Armstrong *et al.* 2004; CEFAS 2005). Similar overlap in the ranges of the winter rings are reported in Baltic cod (Hüssy 2010).

## Manual to ageing of whiting

The otoliths showed a consistent winter ring pattern with decreasing distances between the annuli like in otoliths from Irish Sea and North Sea whiting (Bowers 1954; Gambell & Messtorff 1964) as well as in other gadoids such as hake (Morales-Nin *et al.* 1998) and Baltic cod (Hüssy 2010; Hüssy *et al.* 2010).

CEFAS (2005) provided guidelines for the ageing of North Sea whiting. It was generally recommended to break or section the otoliths, but if read whole the rostrum (i.e. the pointed part of the otolith) is considered the most reliable part of the otolith. In the present study, the post-rostrum or the anterior side of the otolith was found most suitable for ageing of both whole and ground otoliths, as the winter rings were generally difficult to distinguish in the rostrum or the posterior side (fig. 3). Sectioning of the otoliths is not considered the most appropriate method for ageing of whiting as the annuli in older fish become very narrow and may be difficult to distinguish (Gambell & Messtorff 1964), especially in Baltic Sea whiting. Therefore grinding of otoliths is the preferred method. The present study enables reliable ageing of whole otoliths by following the guidelines below.

Based on the results from this study, guidelines for ageing routines of whiting were established:

- Preparation (propylene glycol for 15 min or distilled water for 24 hours to enhance the visibility of the opaque zones)
- Identify visible translucent zones
- Determine whether the edge is opaque or translucent
- Consult the catch date. If the fish was captured
    1) Before January 1st , the translucent edge is not counted
    2) After January 1st , the translucent edge is included in the count
- Measure the distance from the nucleus to the 1st visible translucent zone
    1) If 1500-3400 µm, the translucent zone can be considered as the 1st winter ring
    2) If close to 3600 µm, check the otolith growth trajectory, i.e. measure and compare the distances between the winter rings (c.f. fig. 7 and table 1)
    3) If > 3600 µm, the 1st annulus is likely hidden

  Note: If measuring the distance from the nucleus to the 1st visible translucent zone gives rise to either (2) or (3), grinding or sectioning of the otolith must be performed.

### Future perspectives

The method developed in the present study likely applies to whiting from other areas as well, although the crosscheck, i.e. the maximum distance from the nucleus to the 1st winter ring, may differ somewhat between areas. In this study, a smaller amount of otoliths from whiting inhabiting the North Sea was examined and even though no apparent problem with distinguishing the 1st winter ring existed, some of the otoliths were thick which made it difficult to distinguish all of the winter rings. Nevertheless, decreasing visibility of the 1st annulus has been reported for otoliths from whiting in the North Sea (Gambell & Messtorff 1964), and whether this only applies to some subpopulations inhabiting the area, is yet to be investigated. This emphasizes the usage of the method developed in this study where grinding of the otolith is employed whenever doubt arises. More thorough analyses of whiting otoliths from other areas should be conducted to investigate whether similar or other problems regarding ageing of whole otoliths exist.

The results obtained in this study, together with results from previous ones, emphasize the need for a more holistic approach which incorporates length, catch date overall annulus pattern and application of a crosscheck (maximum distance from the nucleus to the 1st annulus). The stepwise method presented here can be directly implemented in ageing of whole otoliths and should provide correct age estimation, thereby ensuring the reliability and precision of analytical assessment of whiting.

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## Tables

Table 1 Whiting otolith growth. $D_i$ is the distance from the nucleus to the respective winter ring. Mean $D_{i-1}$ is the distance between the 1st and 2nd winter ring (shown in the 2nd winter ring row), 2nd and 3rd winter ring and so forth. All measurements are in µm.

| Winter ring number | Mean D | Range of $D_i$ | Mean $D_{i-1}$ | Range of $D_{i-1}$ |
|---|---|---|---|---|
| Juvenile ring | 1500 | 800-2100 | | |
| 1 | 2600 | 1800-3600 | | |
| 2 | 3900 | 2900-5200 | 1180 | 700-1600 |
| 3 | 5000 | 3800-6400 | 940 | 600-1400 |
| 4 | 6000 | 5200-6900 | 750 | 450-850 |
| 5 | 6400 | 5900-7000 | 650 | 570-680 |

## Figure captions

Fig. 1 Sampling area. ICES subdivisions 22 and 24. The Femern Belt area is encircled.

Fig. 2 Length distribution for the 2009-2011 cohorts. The length distributions for the 2009-2011 cohorts caught in November 2011, January 2012 and May 2012. Estimated numbers are based on the length proportions from a sample taken from each haul, i.e. the number in each length group $i$ for all hauls is calculated as $N_i = \sum S_{i,h} \frac{W_h}{V_h}$, where $S_{i,h}$ denotes the numbers of length group $i$ in the sample drawn from the haul $h$, $W_h$ is the total weight of whiting in the haul $h$ and $V_h$ is the weight of the sample drawn from haul $h$.

Fig. 3 Example of a whole, untreated Baltic Sea whiting otolith. The measurement axis is shown, D = the distance from the nucleus to the 1st annulus.

Fig. 4 Ageing of whole and ground otoliths. Ageing of otolith from a fish, length of 22 cm, caught in May 2012. Image of (a) whole otolith and (b) ground otolith. Nucleus as well as the visual annuli are marked.

Fig. 5 Distance from nucleus to 1st and 2nd annulus as a function of fish length. (a) Distance from the nucleus to the 1st annulus (µm) shown as a function of the fish length (cm) and (b) Distance from the nucleus to the 2nd annulus (µm) shown as a function of the fish length (cm) (NB: the 1st visual annulus of the whole otoliths is plotted as this in reality corresponds to the 2nd annulus). Whole otoliths are shown with black dots and ground otoliths with red triangles.

Fig. 6 Increment width as a function of the distance from the nucleus. Ground otolith from 1-year old fish (length 18 cm) caught in November 2011. (a) Microstructure profile showing the increment widths as a function of the distance from the nucleus. (b) Increment widths of a section of the otolith where the translucent area corresponds to the 1st annulus (7x magnification = 0.32 µm pixel$^{-1}$). (c) $D_{Ground}$ shown with a straight line (≈ 2000µm).

Fig. 7 Otolith growth trajectories. The distances from the nucleus to 1st, 2nd , 3rd and 4th annulus (based on ground otoliths) are shown as a function of the annulus number. The lines show the growth curves for 22 age 3 fish and 10 age 4 fish. Each line corresponds to an individual fish.

Fig. 8 Percentage of otoliths with an opaque edge zone. Otoliths with an opaque edge shown as a percentage of the total number of otoliths analyzed per month

**Figures**

Fig. 1 Sampling area

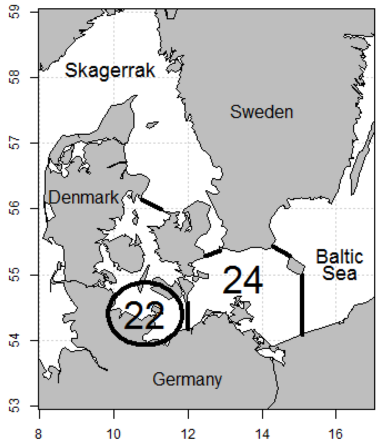

Fig. 2 Length distribution for the 2009-2011 cohorts

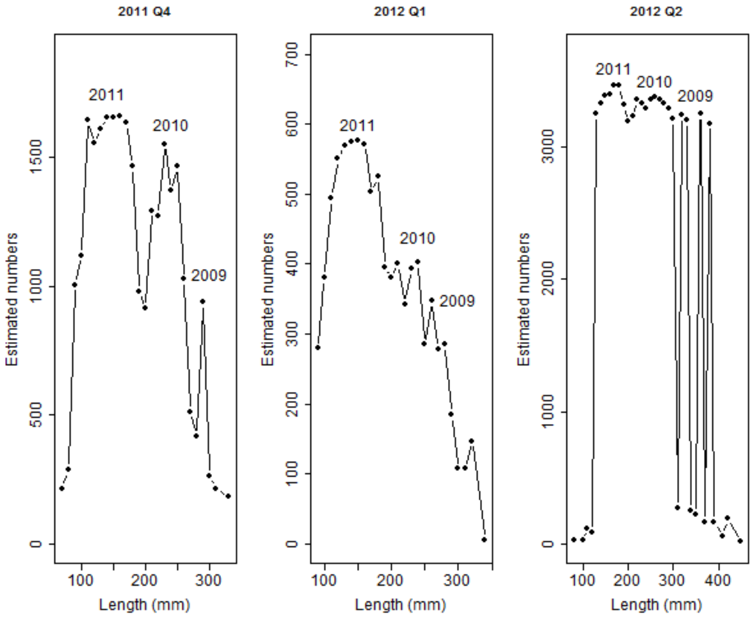

Fig.3 Example of a whole, untreated Baltic Sea whiting otolith

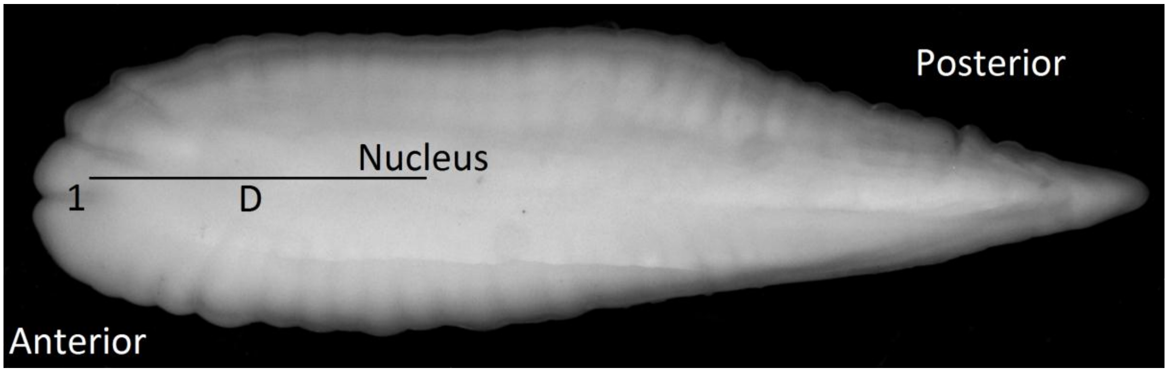

Fig. 4 Ageing of whole and ground otoliths

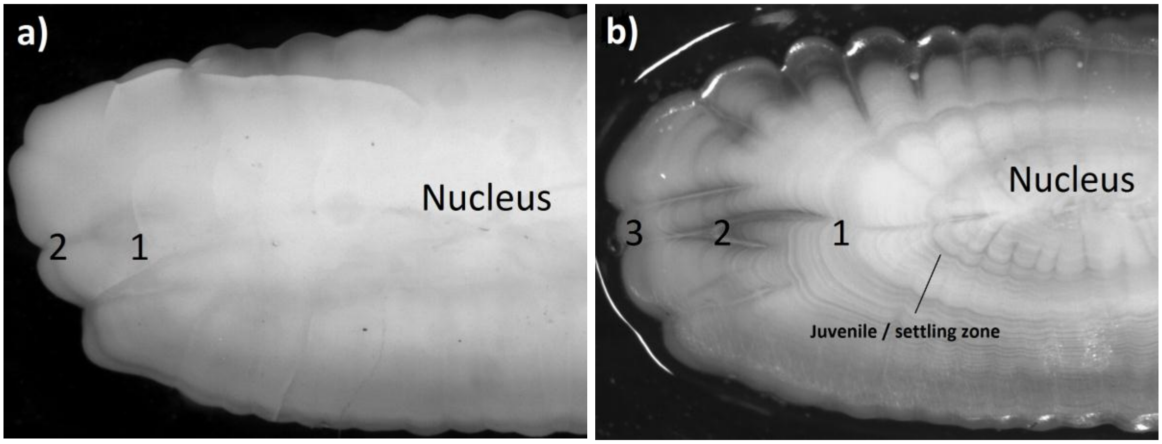

Fig. 5 Distance from nucleus to 1st and 2nd annulus as a function of fish length

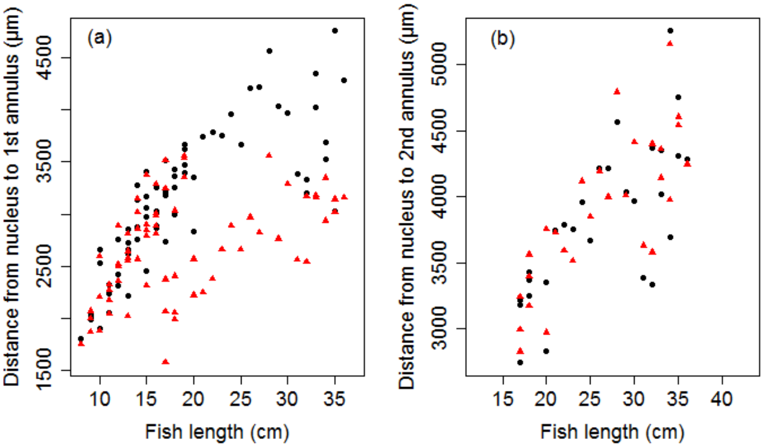

Fig. 6 Increment width as a function of the distance from the nucleus

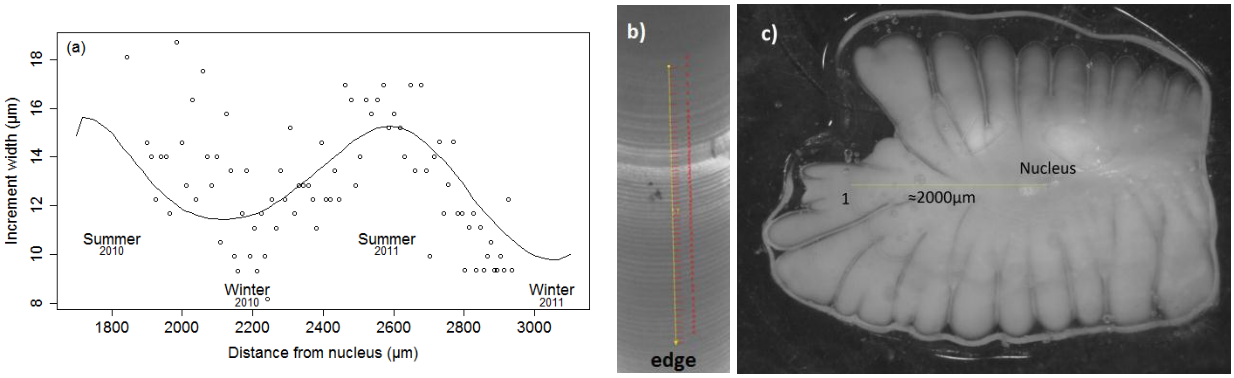

Fig. 7 Otolith growth trajectories

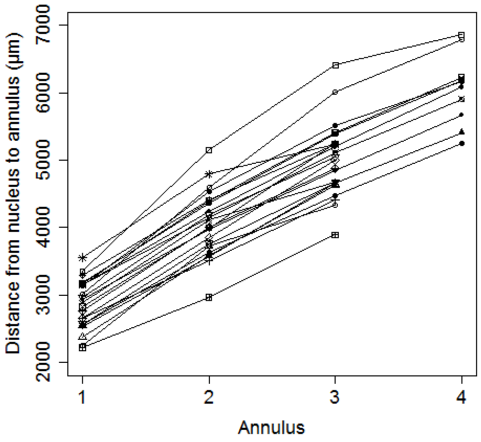

Fig. 8 Percentage of otoliths with an opaque edge zone